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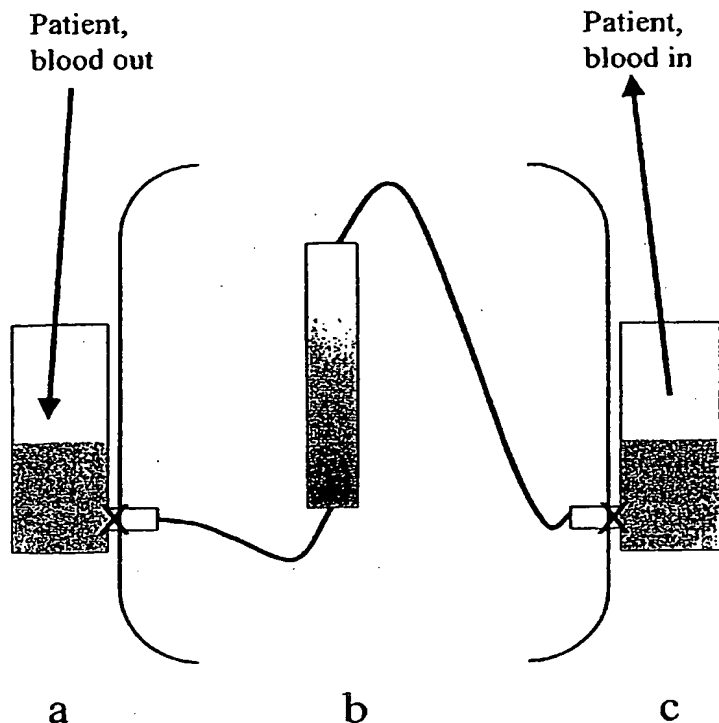
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[Continued on next page]

(54) Title: AN EXTRACORPOREAL STABILISED EXPANDED BED ADSORPTION METHOD FOR THE TREATMENT OF SEPSIS



(57) Abstract: The present invention provides an extracorporeal adsorption method for removing harmful substances from blood in a way that is practicable in everyday clinical practice and applicable for the timely intervention to prevent the development of sepsis. Said extracorporeal adsorption method being effected by an adsorption column assembly where the adsorption column assembly comprising a column and an adsorption medium in the form of particles. The sedimented volume of said particles being at the most 80% of the volume of the column.

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ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO,
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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

Published:

- with international search report
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

AMENDED CLAIMS

[received by the International Bureau on 07 July 2004 (07.07.04);
original claims 2, 13-26 have been replaced by amended claims 2, 13-26.
original claims 1, 3-12 remains unchanged]

1. An extracorporeal adsorption method for removing harmful substances responsible of inducing sepsis caused by Gram-negative in a mammal, said extracorporeal adsorption
5 method being effected by an adsorption column assembly, said adsorption column assembly comprising a column and an adsorption medium in the form of particles, the sedimented volume of said particles being at the most 80% of the volume of the column, said particles being characterised by carrying an affinity specific molecule with a specific affinity for the LPS portion of said Gram-negative bacteria, said method comprising
10 treating blood obtained from said mammal by passing the blood through the adsorption column assembly at such a flow rate that a fluidised bed of the particles is formed.
2. An extracorporeal adsorption method for removing harmful substances responsible of inducing sepsis caused by Gram-negative or Gram-positive bacteria in a mammal, said
15 extracorporeal adsorption method being effected by an adsorption column assembly, said adsorption column assembly comprising a column and an adsorption medium in the form of particles, the sedimented volume of said particles being at the most 80% of the volume of the column, said particles being characterised by carrying an affinity specific molecule with a specific affinity for:
- 20 i) the LPS portion of said Gram-negative bacteria, and/or
ii) Gram-positive bacteria or harmful substances derived from said Gram-positive bacteria,
- 25 said method comprising treating blood obtained from said mammal by passing the blood through the adsorption column assembly at such a flow rate that a fluidised bed of the particles is formed.
3. A method according to claim 1 or 2 wherein the treated blood is capable of being
30 reinfused into the same mammal.
4. A method according to any of claims 1-3, wherein the adsorption column assembly is adapted for fluidised bed adsorption, in particular stabilised fluidised bed adsorption.
- 35 5. A method according to any of the preceding claims, wherein the particles have a density of at least 1.3 g/ml and a mean diameter in the range of 5-1000 μm , such as a density of at least 1.5 g/ml and a mean diameter in the range of 5-300 μm , preferably a density of at least 1.8 g/ml and a mean diameter in the range of 5-150 μm , and most preferred a density of more than 2.5 g/ml and a mean diameter in the range of 5-75 μm .
- 40 6. A method according to any of the preceding claims, wherein the mammal is a human being.

AMENDED SHEET (ARTICLE 19)**BEST AVAILABLE COPY**

7. A method according to any of the preceding claims, wherein the affinity specific molecule is selected from the group consisting of immunoglobulins, peptides, oligonucleotides, receptor proteins, antibiotics, and lectins.
- 5 8. A method according to any of the preceding claims, wherein two or more different affinity specific molecules are present on particles within the adsorption medium.
9. A method according to claim 6 or 7, wherein the affinity specific molecules are selected from immunoglobulins.
- 10 10. A method according to any of the preceding claims, wherein the affinity specific molecule is Polymyxin B.
11. A method according any of the preceding claims, wherein the affinity specific molecule
15 is selected from the group consisting of a Toll-like receptor, most preferably TLR4 or binding fragments thereof or multimeric arrangements thereof, CD14, MD2, TLR2 and LBP, and any combination thereof.
12. A method according to any of the preceding claims, wherein the sedimented volume of
20 the particles is at the most 70% of the volume of the column, such as at the most 60% of the volume of the column, e.g. at the most 50% of the volume of the column.
13. Use of an adsorption medium for the preparation of a therapeutic adsorption column assembly for the continuous therapeutic treatment of sepsis caused by Gram-negative
25 bacteria in a mammal by extracorporeal adsorption, said adsorption column assembly comprising (i) a vessel for continuous obtaining blood from said mammal, (ii) a column comprising the adsorption medium, the sedimented volume of said adsorption medium being at the most 80% of the volume of the column, said adsorption medium being characterised by carrying an affinity specific molecule with a specific affinity for the LPS
30 portion of said Gram-negative bacteria, said column is treating the obtained blood by passing the blood through the adsorption column assembly at such a flow rate that a fluidised bed of the adsorption medium is formed, and (iii) another vessel which continuously delivers blood back to the patient.
- 35 14. Use of an adsorption medium for the preparation of a therapeutic adsorption column assembly for the therapeutic treatment of sepsis caused by Gram-negative or Gram-positive bacteria in a mammal by extracorporeal adsorption, said adsorption column assembly comprising (a) a vessel for continuous obtaining blood from said mammal, (b) a column and the adsorption medium, the sedimented volume of said adsorption medium
40 being at the most 80% of the volume of the column, said adsorption medium being characterised by carrying an affinity specific molecule with a specific affinity for:
- 1) the LPS portion of said Gram-negative bacteria, and/or

AMENDED SHEET (ARTICLE 19)

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- ii) Gram-positive bacteria or harmful substances derived from said Gram-positive bacteria,
said column is treating the obtained blood by passing the blood through the adsorption
column assembly at such a flow rate that a fluidised bed of the adsorption medium is
5 formed, and
- (c) another vessel which continuously delivers blood back to the patient.
15. The use according claim 13 or 14, wherein the flow rate of the blood through the
10 column assembly is such that expansion ratio of the fluidised bed is at least 1.3, such as at
least 1.5.
16. The use according to any of the claim 12-15, wherein the steps (a), (b) and (c) are
preceded by a initial step by which a substance is first injected into the blood stream of the
15 mammal.
17. The use according to any of the claims 13-16, wherein the mammal is a human being.
18. The use according to any of the claims 13-17, wherein the particles have a density of
20 at least 1.3 g/ml and a mean diameter in the range of 5-1000 μm , such as a density of at
least 1.5 g/ml and a mean diameter in the range of 5-300 μm , preferably a density of at
least 1.8 g/ml and a mean diameter in the range of 5-150 μm , and most preferred a
density of more than 2.5 g/ml and a mean diameter in the range of 5-75 μm .
- 25 19. A use according to any of claims 11-18, wherein the stabilised fluidised bed is placed in
line with a switch capable of being activated when a blood substance reaches a pre-set
value, said blood substance is monitored by a device, said device is placed in line with the
blood circulation, said device sending the activating signal to the switch when said value is
reached.
- 30 20. The use according to any of the claims 13-19, wherein the affinity specific molecule is
selected from the group consisting of immunoglobulins, peptides, oligonucleotides,
receptor proteins, antibiotics, and lectins.
- 35 21. The use according to any of the claims 13-20, wherein two or more different affinity
specific molecules are present on particles within the adsorption medium.
22. The use according to claim 20 or 21, wherein the affinity specific molecules are
selected from immunoglobulins.
- 40 23. The use according to any of the claims 20 or 21, wherein the affinity specific molecule
is Polymyxin B.

AMENDED SHEET (ARTICLE 19)

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24. A use according to claims 13-23, wherein the affinity specific molecule is selected from the group consisting of a Toll-like receptor, most preferably TLR4 or binding fragments thereof or multimeric arrangements thereof, CD14, MD2, TLR2 and LBP, and any combination thereof.

5

25. The use according to any of the claims 13-24, wherein the sedimented volume of the particles is at the most 70% of the volume of the column, such as at the most 60% of the volume of the column, e.g. at the most 50% of the volume of the column.

10 26. The use according to any of the claims 13-25, wherein the flow rate is such that stabilised fluidised bed of the particles is formed.

AMENDED SHEET (ARTICLE 19)

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INTERNATIONAL SEARCH REPORT

International Application No.

PCT/DK 03/00483

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 B01D15/00 G01N33/50 B01J8/18

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 B01D G01N B01J C07K A61M

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP 1 057 529 A (TORAY INDUSTRIES) 6 December 2000 (2000-12-06) abstract	4-26
A	page 2	1-3
Y	--- JAN FEUSER ET AL: "Cell/adsorbent interactions in expanded bed adsorption of proteins" BIOSEPARATION, vol. 8, 1999, pages 99-109, XP002259859 abstract	4-26
A	---	1-3
Y	US 5 837 826 A (ROBICHAUD MICHAEL J ET AL) 17 November 1998 (1998-11-17) abstract	1,4-13, 15,17-26
A	column 1, line 49 -column 2, line 12 column 2, line 60 - line 64 ---	2,3,14
	--- -/--	

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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Date of the actual completion of the international search

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Målin Söderström

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/DK 03/00483

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 6 090 292 A (OTTO VEIT ET AL) 18 July 2000 (2000-07-18) abstract	1,4-13, 15,17-26
A	column 4, line 60 - line 64; figure 1 ---	2,3,14
A	EP 0 955 312 A (SEIKAGAKU KOGYO CO LTD) 10 November 1999 (1999-11-10) summary, page 10, line 50 - page 11, line 53 ---	1-26
P,A	WO 02/053251 A (LIHMES APS ;LIHME ALLAN OTTO FOG (DK)) 11 July 2002 (2002-07-11) abstract; figures 1-5 -----	1-26

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK/93/00483

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 13, 14
because they relate to subject matter not required to be searched by this Authority, namely:
see FURTHER INFORMATION sheet PCT/ISA/210
2. ☒ Claims Nos.: 1-26
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1. 3-15. 17-26.

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Claims Nos.: 13, 14

Claims 13 and 14 relates to methods of treatment of the human or animal body by surgery or by therapy or diagnostic methods practised on the human or animal body (PCT Rule 39.1(iv)), due to the sentence "obtaining blood from said mammal". Nevertheless, a search has been executed for these claims. The search has been based on the alleged effects of the compounds or compositions.

Continuation of Box I.2

Claims Nos.: 1-26

Claim 16 does not meet the requirements of Article 6 PCT in that the matter for which protection is sought is not clearly defined. Claim 16 refers to steps (a), (b), and (c) in claims 12-15, which does not comprise these steps. It is not clear from claim 16 or the description why a substance is injected in the blood system or which substance is injected.

Following separate inventions have been identified:

1. Claims 1, 3-15, 17-26. An extracorporeal adsorption method for removing harmful substances using a column with adsorption medium in form of particles forming a fluidised bed. The particles carry an affinity specific molecule having affinity for the LPS portion of Gram-negative bacteria and/or Gram-positive bacteria or harmful substances derived from said Gram-positive bacteria.

2. Claims 2, 3, 5-12, 19-25. An extracorporeal adsorption method for removing harmful substances using a column with adsorption medium in form of particles. The particles carry an affinity specific molecule having affinity for the LPS portion of Gram-negative bacteria and/or Gram-positive bacteria or harmful substances derived from said Gram-positive bacteria.

D1 represents the most relevant prior art. From D1 is known a method purifying blood by using a column having particles with affinity to endotoxin of gram-negative bacteria, e.g. lipopolysaccharide from E.coli, see fig 2, column 4, line 61, claims 1-11. Therefore the common special technical feature, the particles carry an affinity specific molecule having affinity for the LPS portion of Gram-negative bacteria, for the two inventions are known.

Invention 1 and 2 are therefore not linked with a common special technical feature. Hence the requisite unity of invention (Rule 13.1 PCT) therefore no longer exists inasmuch as a technical relationship involving

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

one or more of the same or corresponding special technical features in the sense of Rule 13.2 PCT does not exist between invention 1 and 2. Thus, the invention lacks unity *0* posteriori.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1, 3-15, 17-26

Claims 1, 3-15, 17-26. An extracorporeal adsorption method for removing harmful substances using a column with adsorption medium in form of particles forming a fluidised bed. The particles carry an affinity specific molecule having affinity for the LPS portion of Gram-negative bacteria and/or Gram-positive bacteria or harmful substances derived from said Gram-positive bacteria.

2. Claims: 2, 3, 5-12, 19-25

Claims 2, 3, 5-12, 19-25. An extracorporeal adsorption method for removing harmful substances using a column with adsorption medium in form of particles. The particles carry an affinity specific molecule having affinity for the LPS portion of Gram-negative bacteria and/or Gram-positive bacteria or harmful substances derived from said Gram-positive bacteria.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/DK 03/00483

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
EP 1057529	A	06-12-2000	AU 1689900 A	12-07-2000
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			EP 1345666 A1	24-09-2003

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